REMARKS

The present invention provides 1-aryl- or 1-alkylsulfonylheterocyclylbenzazole compounds of formula I, and the therapeutic use thereof for the treatment of CNS disorders related to or affected by the 5-hydroxytryptamine-6 receptor.

Claims 1-18 are pending in this application. New claims 19-23 have been added to clearly express one embodiment of the invention. New claims 19-23 describe a subset of the genus of original claim 1 and fall within the scope of claim 1. Applicants believe no new matter has been added by this amendment.

1. The Examiner has required restriction of the claims under 35 USC §121 as follows:

I. Claims 1(in part), 3-4 (in part), 7-12 (in part), and 18 (in part), drawn to a compound of formula I wherein A is C, CR_{10} or N; and m is an integer of 1;

II. Claims 1(in part), 3-4 (in part), 7-12 (in part), and 18 (in part), drawn to a compound of formula I wherein A is C or CR_{10}; and m is an integer of 2;

III. Claims 1(in part), 3-4 (in part), 7-12 (in part), and 18 (in part), drawn to a compound of formula I wherein A is C or CR_{10}; and m is an integer of 3;

IV. Claims 1(in part), 2, 3-4 (in part), 5-6, 7-12 (in part), 13, 14-15 (in part), 16, 17 and 18 (in part), drawn to a compound of formula I wherein A is N; and m is an integer of 2; and

V. Claims 1(in part), 3-4 (in part), 7-12 (in part), 13-15 (in part), and 18 (in part), drawn to a compound of formula I wherein A is N; and m is an integer of 3.

Applicants respectfully traverse the foregoing restriction and solely in compliance with the provisions of 37 CFR §1.143, Applicants hereby elect with traverse Group IV, claims 1(in part), 2, 3-4 (in part), 5-6, 7-12 (in part), 13, 14-15 (in part), 16, 17 and 18 (in part), drawn to a compound of formula I wherein A is N; and m is an integer of 2. Applicants reserve the right to file a divisional application directed to the non-elected subject matter.

2. Per the Examiner's request, the abstract of the present application has been amended to delete the title of the invention and to define the variables of A, X, Y and m. Applicants believe the abstract as amended is not defective and conforms to the requirements of the MPEP § 608.1(b).

3. Claims 1, 3-4, 7-12 and 18 have been objected to by the Examiner as being drawn to multiple inventions for the reasons set forth in the requirement for restriction.

Claims 1, 3-4, 7, 10, 12 and 18 have been amended to exclude the non-elected subject matter. Claims 8, 11 and 13 have been cancelled. Applicants reserve the right to file a divisional application directed to the non-elected subject matter. In view of the above amendment, applicants respectfully request the Examiner to reconsider and withdraw his objection.

4. Claims 7, 8, 10 and 11 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention. The Examiner contends that the method of claim 7 drawn to CNS disorders related to or affected by the 5-HT6 receptor is unduly broad and lacks sufficient enablement in the specification for one of ordinary skill in the art.

Applicants respectfully traverse the rejection. Claim 7 has been amended to embrace the limitations of claims 9 and 10. Claims 8 and 11 have been cancelled. The role of the 5-HT6 receptor site, and the compounds which bind to said site, in CNS diseases is well established in the art, see attached (A) Behavioural Brain Research, 118 (2001), 107-110; (B) Expert Opinion Therapeutic Patents (2002) 12(4), 513-527; and (C) Current Opinion in Investigational Drugs, (2001) 2(1), 118-122. The modulaton of the 5-HT-6 receptor site is clearly linked to treatment of cognitive dysfunction (A, p. 109; B, p.525 and C, p. 120) and neurodegenerative disease (C, p. 120). The direction of guidance of the specification is toward one of ordinary skill in the art of small molecule pharmaceuticals. The chemical and biological examples of the present application clearly teach how to make and use the formula I compound. Evidence of "structurally similar" compounds in the art is not a requirement of 35 U.S.C. § 112, first paragraph. The fact that the Examiner finds no evidence in the art that a compound structurally similar to the formula I compound of the present application acts as a 5-HT6 ligand or is useful for the treatment of CNS disorders is supportive of the patentability of the present application. It is surprising that the formula I compound demonstrates a high affinity for the 5-HT6 receptor. Moreover, enablement for this affinity is clearly supported in the comparative biology Example 103 of the present application wherein 77% of the formula I compounds tested demonstrated equal to or greater than the 5-HT6 binding affinity of the known pharmaceutical compounds used as test standards. The standards used in Example 103, besides having a known affinity for the 5-HT6 receptor, have been indicated for use in the treatment CNS diseases such as

Parkinson's disease, schizophrenia, migraine, depression and psychosis. Additionally, the test compounds used in Example 103 demonstrated high selectivity for the 5-HT6 receptor site, increasing the correlation to treatment of CNS disorders affected by the 5-HT6 receptor. It is clear that out of all of the possible compounds in the universe known to be available, or known to be able to be synthesized, (truly an infinite number), the select and particular compounds of the present application demonstrate predictable utility in the modulation of the 5-HT6 receptor site and, correspondingly, in the treatment of CNS disorders relating thereto. In support of that correspondence and in support of the level of skill of those in the art, please see attached representative references A, B and C. Considering the serious nature of CNS disorders and the lack of effective treatment therefor, one of ordinary skill in the art would not consider the teachings of the present application to require undue experimentation. In sharp contrast, the skilled artisan would consider the need for experimentation to be greatly diminished by the teachings of the present application.

5. Claims 10 and 11 are rejected under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention. The Examiner contends that the specification is not enabled for the method of use claim 10, i.e. a method of treating a neurodegenerative disorder.

Applicants respectfully traverse the rejection. Claim 10 has been amended to embrace the scope of claim 11. Claim 11 has been cancelled. Applicants believe the scope of amended claim 10 is supported by the specification and claims as originally filed. Amended claim 10 is directed to the treatment of two specific cognitive disorders, Alzheimer's disease and Parkinson's disease. Support for the affinity of the formula I compound for the 5-HT6 receptor site is found in Example 103 of the specification and the corresponding use of compounds which bind to the 5-HT6 receptor for treating cognitive dysfunction has been demonstrated in the literature, see attached reference C, p. 119 and 120, wherein a 5-HT6 ligand was put into phase I clinical trials based upon the evidence that said ligand may "be very valuable for treating cognitive abnormalities in schizophrenia and neurodegenerative diseases". Applicants believe amended claim 10 meets all of the requirements of 35 U.S.C. § 112, first paragraph.

6. Claims 1-18 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner questions the use of the term "optionally substituted" in

claim 1 or elsewhere in the claims. The Examiner also feels the term "cycloheteroalkyl" is not clear. In claim 7, the Examiner contends the scope of a disorder of the central nervous system related to or affected by the 5-HT6 receptor is unknown, i.e. the response of a given CNS disease to the modulation of the 5-HT6 receptor is unknown.

Applicants respectfully traverse the rejection. The phrase "optionally substituted" is clearly defined in the detailed description of the specification on page 6, lines 20-26 and on page 7, lines1-15. The term "cycloheteroalkyl" is clearly defined in the detailed description of the specification on page5, lines 26-31 and on page 6, lines 1-5. These definitions are very explicit and particularly point out the meaning of the terms "optionally substituted" and "cycloheteroalkyl" as they are used in the specification and claims of the present application. Claim 7 has been amended to recite the specific central nervous system disorders included in the treatment method. There is substantial evidence in the literature to support the response of the specific diseases, recited in amended claim 7, to a 5-HT6 ligand. Applicants believe amended claims 1-18 fulfill the requirements of 35 U.S.C. § 112, second paragraph.

In conclusion, Applicants believe that the Examiner's objection to the claims and all of the rejections under 35 U.S.C. § 112, first and second paragraphs have been overcome in view of the foregoing and in view of the amendments to the claims, as shown hereinabove. Applicants respectfully request the Examiner to enter the above amendments, consider the above remarks, withdraw the rejections and allow the application.

Attached hereto are a page captioned "List of Attached References" and copies of the listed references, which have been discussed hereinabove.

Favorable treatment of the application is earnestly solicited.

Respectfully Submitted,

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Short communication

Effects of the 5-HT_6 receptor antagonist Ro 04-6790 on learning consolidation

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Abstract

The 5-HT_6 receptor antagonist Ro-04-6790 or 8-OH-DPAT injection improved learning consolidation on an autoshaping task, while mCPP, scopolamine and dizocilpine decreased the performance. The effect induced by scopolamine, but not that induced by mCPP, was reversed completely by Ro-04-6790, while dizocilpine effect was antagonized partially. Nevertheless, ritanserin or WAY 100635, but not Ro 04-6790, antagonized the 8-OH-DPAT facilitatory effects on learning consolidation. As WAY 100635 did not modify the Ro 04-6790 facilitatory effect, hence 5-HT_{1A}, and/or 5-HT_7, but not 5-HT_6, receptors might mediate the 8-OH-DPAT facilitatory effect on learning consolidation. Since, the Ro 04-6790 facilitatory effect was unaffected by 5-HT_{1A}, 5-$HT_{2A/2B/2C}$, 5-HT_3 or 5-HT_4 receptor blockade, thereby, the facilitatory effect induced by Ro 04-6790 involved specifically 5-HT_6 receptors. Indeed, the present data provide further support to the notion that, 5-HT_6 receptors play a significant part in the learning consolidation under normal and dysfunctional memory conditions.

Keywords: Serotonin; Receptors; 5-HT; Learning; Autoshaping; Rat

1. Introduction

Serotonergic neurotransmission involves multiple 5-hydroxytryptamine (5-HT) receptor subtypes (5-HT_1 to 5-HT_7) [1]. Among these, the 5-HT_6 and 5-HT_7 receptors show a regional distribution within the central nervous system [1,4,8], in areas, which have been associated with learning and memory processes (see [13,14], for reviews). Diverse physiological and/or behavioral effects following manipulation of 5-HT_6 receptors have been reported [1,4,9]. For instance, 5-HT_6 antisense oligonucleotide decreased 5-HT_6 gene expression and the induced behaviors by antisense treatment were antagonized by atropine (a muscarinic antagonist) [3]. Rats 5-HT_6 antisense treatment produced an enhanced spatial learning acquisition in the water maze [2]. It should be noticed that, the studies mentioned above were based upon the sole use of nonselective either, agonist or antagonist drugs for 5-HT_6 receptor. Nevertheless, a growing interest for the 5-HT_6 receptors as target by the drugs development useful for psychiatric disorders treatment [9], such as schizophrenia. Interestingly, an association between the 5-HT_6 receptor polymorphism C267T and Alzheimer's disease (AD) has been reported [20]. Indeed, two novel, presenilin 1 (PS1) and presenilin 2 (PS2) have been implicated casually in the pathogenesis of AD and more than 50 misense mutations of PS1 are known [5], including the polymorphism C267T. Accordingly, drugs acting at 5-HT_6 receptors could modulate learning and memory. Hence, in the current work, it was decided to study the effect of the 5-HT_6 antagonist Ro 04-6790 [4], the 5-$HT_{1A/7}$ agonist 8-OH-DPAT, the 5-HT_{1A} antagonist WAY100635, the 5-$HT_{1A/1B/1D/2A/2C/7}$ agonist/antagonist mCPP, the 5-HT_{2A} antagonist ketanserin, the 5-$HT_{2A/2C/6/7}$ antagonist ritanserin, the 5-HT_3 antagonist ondansetron, the 5-HT_4 antagonist GR125487 [1,10], scopolamine (an anticholinergic), dizocilpine (an

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NMDA antagonist) and phenserine (an AChE inhibitor) [13] on associative learning. An autoshaping test was used here, which had shown to be useful to study learning and memory changes produced by age and/or drugs (see [13,18]). Importantly, whether drugs are administered before training, results very difficult to determine whether these act on memory or other processes (e.g. attention, motivation, locomotor activity, etc.; [13]; for recent review, see [11]); however, the post-learning treatment strategy allows to exclude most of the problems that the former strategy presents, i.e. producing changes on attention, motivation, locomotor activity, etc.; unrelated with learning and memory per se.

2. Methods

Adult male Wistar rats were housed collectively in a temperature- and light-controlled room under a 12-:12-h light-dark cycle (light on at 7:00 h). Water and food were provided ad libitum for a week. After that period, body weights were reduced to 85% by gradually reducing the food intake during 7 days.

2.1. Autoshaping training

Each rat was placed in an experimental chamber and allowed to habituate to the experimental environment until the animal found and ate 50 food pellets (each pellet 45 mg). Immediately afterwards, the program began. This consisted in the presentation of a retractable illuminated lever for 8 s (conditioned stimulus, CS), followed by delivery of a food-pellet (unconditioned stimulus, US) every 60 s. When the animal pressed the CS, the lever was retracted, the light was turned off, and a food pellet (US) was delivered immediately; this was defined as a conditioned response (CR). The increase or decrease in percentage of CR was considered as an enhancement or impairment in learning, respectively. The first session consisted of 10 trials and the second session of 20. All compounds were injected immediately following the first autoshaping session; then, the sessions test was performed 24 h later and, in other groups, the animals were given Ro 04-6790 immediately after the first autoshaping session and, 10 min later, the animals received scopolamine or dizocilpine and 24 h later the session test. The data displayed correspond to the session test (i.e. second session) [13].

2.2. Drugs

The drugs used in the present study were: Ro 04-6790 (4-amino-*N*-[2,6-bis(methylamino)-4-pyrimidinyl]-benzenesulfonamide dihydrochloride) (RBI-Sigma, Saint Louis, MO, USA); scopolamine HBr, dizocilpine maleate, 8-OH-DPAT ((±)-8-hydroxy-2-(di-n-propilamino) tetralin HCl), WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexanene carboxamide 6trihydrochloride), mCPP (1-(3-chlorophenyl)piperazine dihydrochloride), ketanserin tartrate, and ritanserin (Research Biochemical Inc., Wayland, MA, USA); ondansetron and GR125487 (Glaxo-Wellcome, UK), and phenserine (Gerontology Research Center, NIH, Baltimore, MA, USA). All drugs were injected intraperitoneally (i.p.) and dissolved in saline solution or methylcellulose in a volume of 1 ml/kg. It should be noticed that, all drugs used here, but Ro 04-6790, have their respective full dose–response curves reported previously [13–16].

Operant chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) for rats with standard sound-attenuation were used. Chambers were 25-cm wide, 29-cm long, and 25-cm high. A retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever required 10 g F to operate. A food magazine for rat pellets (Bio Serv, Frenchtown, NJ, USA) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner. Solid-state programming equipment was used for control and recording (Coulbourn Instruments, Lehigh Valley, PA, USA).

2.3. Data acquisition and statistical analysis

As in previous works [13–18], the responses (CR) in the CS presence were divided by the trials in session, expressed as a percentage of the total trials, and analyzed using analysis of variance (ANOVA) followed by additional post-hoc comparisons using the Tukey test. In all comparisons, $P < 0.05$ was used as criterion for significance. The n was eight per group, and animals were used only once.

3. Results

As depicted in Table 1, the Ro 04-6790 administration increased significantly the CR% [$F(4,39) = 6.2$; $P < 0.05$], and this effect was significant at the 5 mg/kg dose. As reported previously [16], 8-OH-DPAT, significantly [$F(4,39) = 3.5$; $P < 0.05$] increased the CR%. At the tested doses, neither WAY100635, ketanserin, ritanserin, ondansetron, nor GR125487 by itself, affected the CR percentage of mCPP [$F(6,55) = 1.0$; $P > 0.05$], [$F(6,55) = 1.3$; $P > 0.05$]. In contrast, scopolamine [$F(3,31) = 3.9$; $P < 0.05$] or dizocilpine [$F(3,31) = 4.9$; $P < 0.05$], but not phenserine[$F(3,31) = 1.2$; $P > 0.05$] administration decreased significantly the CR% (Table 1). Nevertheless, Ro 04-6790 blocked the scopolamine or dizocilpine impairment-effect, but not that induced

Table 1
Effects of various 5-HT receptor drugs on the percentage of conditioned response (CR) in an autoshaping learning task

Treatment (mg/kg)	CR (%)
Control	10 ± 2
Ro 04-6790 (1)	12 ± 5
Ro 04-6790 (5)	26 ± 7[a]
Ro 04-6790 (10)	29 ± 10
8-OH-DPAT (0.062)	29 ± 5[a]
Control	10 ± 2
WAY 100635 (0.01)	11 ± 3
mCPP (5)	2 ± 2[a]
Ketanserin (0.001)	16 ± 3
Ritanserin (0.1)	17 ± 4
Ondansetron (0.01)	17 ± 5
GR 125487 (0.78)	9 ± 4
Control	12 ± 3
Scopolamine (0.17)	3 ± 1[a]
Dizocilpine (0.1)	2 ± 1[a]
Phenserine (0.5)	19 ± 6

[a] Values are significantly different from control-vehicle ($P<0.05$ by Tukey test).

by mCPP (Table 2). Moreover, WAY 100635, ketanserin, ondansetron or GR 125487 had no effect by itself and did not modify Ro 046790 effect (Table 2). Ritanserin had no effect by itself, however, significantly $[F(4,39) = 3.9;\ P < 0.05]$ antagonized the increase of CR% induced by Ro 04-6790 (Table 2). As previously reported [16] the 8-OH-DPAT facilitatory effect was antagonized by WAY 100635 and ritanserin [17,18], but not significantly affected Ro 04-6790 (the current work).

Table 2
Effects of various 5-HT receptor drugs on the percentage of conditioned response (CR) in an autoshaping learning task[a]

Treatment (mg/kg)	CR (%)
Control	10 ± 2
Ro 04-6790 (5)	26 ± 7*
Ro 04-6790 (5) + 8-OH-DPAT (0.062)	16 ± 5+
Ro 04-6790 (5) + WAY 100635 (0.01)	31 ± 3*
Ro 04-6790 (5) + Ketanserin (0.001)	28 ± 3**
Ro 04-6790 (5) + Ritanserin (0.1)	17 ± 3+
Ro 04-6790 (5) + Ondansetron (0.01)	27 ± 5*
Ro 04-6790 (5) + GR 125487 (0.78)	31 ± 4*
Control	11 ± 3
WAY 100635 (0.1) + 8-OH-DPAT (0.062)	16 ± 5+
Ro 04-6790 (5) + mCPP (5)	6 ± 2+
Ro 04-6790 (5) + Scopolamine (0.17)	16 ± 3+
Ro 04-6790 (5) + Dizocilpine (0.1)	9 ± 5*

[a] *, Values are significantly different from the control-vehicle or, +, 5-HT antagonist control group ($P<0.05$ by Tukey test).

4. Discussion

Inasmuch as, in the present study, the animals received the drugs after the first training session and once they had the opportunity to learn where to find food-pellets, and thus excluding nonspecific change [11]. Therefore, these data reflect an effect in the learning consolidation [11,13,14]. The major finding of the present study was that, post-training injection of the 5-HT_6 receptor antagonist Ro 04-6790 alone enhanced learning consolidation. While, ritanserin or WAY 100635 had no effect; the 8-OH-DPAT facilitatory effect was nevertheless, completely or partially (but not significantly) antagonized by WAY 100635 or Ro 04-6790, respectively, suggesting that 5-HT_{1A}, 5-HT_6 and 5-HT_7 receptors could not interacting during learning consolidation. The Ro 04-6790 facilitatory effect on learning consolidation was unaffected by WAY 100635 (a 5-HT_{1A} antagonist), ketanserin (a 5-HT_{2A-2c} antagonist), ondansetron (a 5-HT_3 antagonist), or GR 125487 (a 5-HT_4 antagonist), but reversed by ritanserin (a 5-$HT_{2A-2C/6/7}$ antagonist) [1,4]. Hence, it seems logical to conclude that, 5-HT_6 receptors are specifically mediating the Ro 04-6790 facilitatory effects. Moreover, 5-HT_6 receptor blockade did not affect the mCPP (a 5-$HT_{1A/1B/1D/2A/2C/7}$ agonist/antagonist) impairment-induced effect, nevertheless, Ro 04-6790 completely (scopolamine) or partially (dizocilpine) normalized a poor memory. Moreover, AChE inhibition modified weakly the Ro 04-6790 facilitatory effect, suggesting that 5-HT_6 receptor may be affecting the cholinergic neurotransmission directly. Operational (i.e. pharmacological) information [1,13,14,19] regarding 5-HT_{1A} and/or 5-HT_7 receptors supports the conclusion that these receptors manipulation did not alter significantly the Ro 04-6790 facilitatory effects on learning consolidation.

The present findings are consistent with emerging evidence indicating that 5-HT_6 receptors participation on cognitive processes, particularly in learning and memory (see [4], for review). For example, central administration of antisense oligonucleotides targeted for the 5-HT_6 receptors had no effect in visual acuity or swim speed in rats nonetheless, they produced a facilitated performance in the water maze [2]. Similarly, the highly brain penetrant 5-HT_6 receptor antagonist SB-271046 [19] improved retention in the water maze and, produced a significant performance improvement of aged rats in an operant-delayed alternation task. In this connection, it is noteworthy that, systemic administration of other 5-HT_6 receptor antagonists, SB-271046, produced a significant tetrodotoxin-dependent, increase in extracellular levels of glutamate and aspartate within the frontal cortex [6]. More importantly, 5-HT_6 receptors occur in hippocampus, amygdala, and several cortical layers [1,4], brain areas involved in learning and

memory [13]. And the 5-HT_6 267C allele gene has been reported as a risk factor for Alzheimer's disease [20]. Altogether, this information provides additional support to physiological, pathophysiological and therapeutic roles of 5-HT systems in learning and memory (see [13,14], for reviews).

Cognitive function, particularly learning and memory, is impaired markedly in most schizophrenics patients and atypical antipsychotics drugs pharmacologically related to clozapine and 5-HT_{2A} receptor antagonism, may improve cognitive dysfunction [12]. Indeed, in support to this contention, it has been suggested [17,18] that 5-$HT_{2A}/_{2B}/_{2C}$ receptors blockade may provide some benefit to reverse poor learning consolidation conditions associated with serotonergic, cholinergic and/or glutamatergic neurotransmission, such as those found in AD patients, as well as target for novel antipsychotics. However, clozapine is a high-affinity antagonist at both human and rat $5HT_6$ receptors [4], and considering the present findings and the above-mentioned evidence, it could hence reasonable to not exclude either 5-HT_{2A} and 5-HT_6 receptors potential involvement in clozapine-like drugs in learning and memory studies. Of course, future studies must also explore the effects of agents such as the 5-HT_6 receptor agonist 2-ethyl-5-methoxy-*N*, *N*-dimethyltryptamine and its 2-ethyl substituent with a phenyl group, which retains 5-HT_6 receptor affinity but lacks agonist character [7]. The latter compound could represent a 5-HT_6 receptor inverse agonist.

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Expert Opinion

Monthly Focus: Central & Peripheral Nervous Systems

Recent progress in 5-HT_6 receptor antagonists for the treatment of CNS diseases

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In light of the barrage of recent reviews on 5-HT_6 receptor antagonists, this article highlights and reviews the research advances published in patent literature between January 1998 and December 2001. The article is supplemented with selected references on design, synthesis and development of novel 5-HT_6 agents to treat CNS diseases and to study and understand their mechanism and pathophysiology. Emphasis is given to recent advances in the possible involvement of 5-HT_6 serotonergic agents in the treatment of schizophrenia and depression. By no means has any attempt been made to exhaustively review the literature but rather, primary references along with citations to recent literature reviews have been included in each section.

Keywords: 5-HT_6, agonists, antagonists, cognitive, depression, dysfunction, potency, schizophrenia, selectivity, side effects

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1. Introduction

The discovery of several antipsychotic agents (notably clozapine 1, olanzapine 2 and seroquel 3) and antidepressants (clomipramine 4, amitriptyline 5, doxepin 6 and nortrypyline 7) as highly potent 5-HT_6 receptor antagonists [1-3] has led to acceleration in research efforts toward finding more potent and selective 5-HT_6 receptor antagonists. The high 5-HT_6 receptor affinity of these therapeutically important antipsychotics and antidepressants seems to suggest a possible role for this receptor, and hence 5-HT_6 antagonism, in the treatment of schizophrenia and depression. Considerable advances have been made in the research and discovery of more potent and selective 5-HT_6 receptor antagonists since the patent literature was last reviewed in 1998 [4]. More intriguing is the increased understanding of the mechanism and pathophysiology of 5-HT_6 antagonism in the treatment of CNS diseases. The importance of 5-HT_6 antagonists is manifested by the growing numbers of patents filed and scientific papers published in recent years. These efforts have yielded highly potent and selective ligands to target relevant receptor subtypes in the treatment of CNS diseases.

5-HT (serotonin), a key neurotransmitter of the CNS and PNS, has been implicated in a variety of sensory, motor and behavioural processes [1]. Diverse effects of this neurotransmitter are related to the extensive projections of serotonergic neurons throughout the brain and large number of distinct serotonin receptor subtypes. At least 14 distinct serotonin receptor subtypes are expressed in the mammalian CNS [8,9]. These receptors have been classified into seven main families: 5-HT_{1-7}. The 5-HT_1 family comprises subtypes 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F}; the 5-HT_2 family consists of subtypes 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} and the 5-HT_5 family consists of subtypes 5-HT_{5A} and 5-HT_{5B}. During the last four years, ~ 90% of patent applications citing CNS diseases claim seratonergic

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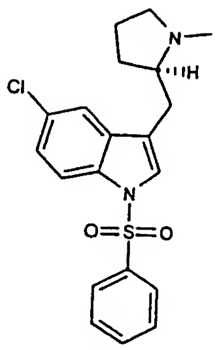

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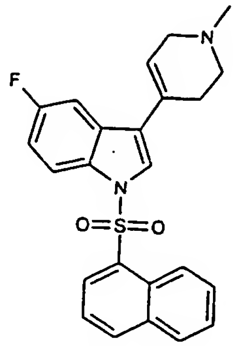

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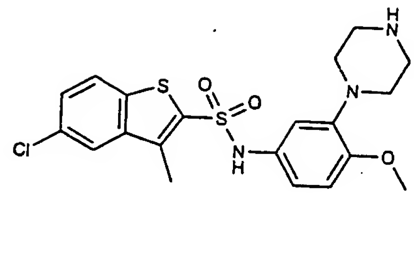

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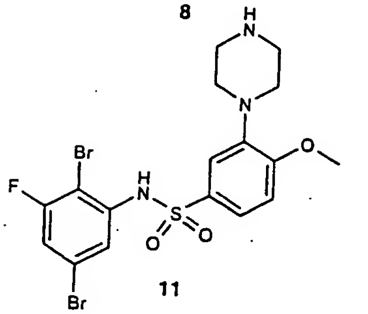

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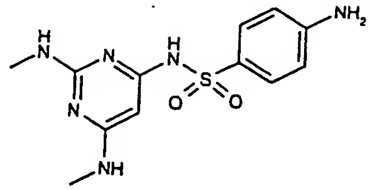

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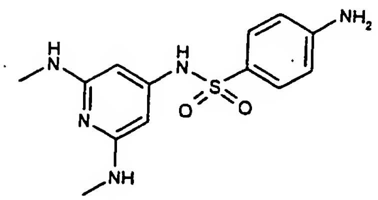

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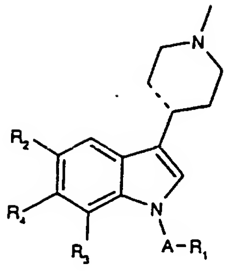
with schizophrenia and Alzheimer's disease (AD) [16]. In addition, association between the good response to clozapine and the T/T genotype has been reported. To test this hypothesis, this polymorphism was genotyped in two independent samples of clozapine-treated patients and a sample of olanzapine-treated patients, including responders and non-responders. Preliminary results of the olanzapine study show no association between this polymorphism and olanzapine response, although the results follow the same trend as that of clozapine. A stratified analysis of both clozapine samples showed a slight association between the polymorphism and clozapine response genotypes (p = 0.05) and alleles (p = 0.02). It has been concluded that these results provide further evidence to suggest that the 5-HT$_6$ 267-C/T polymorphism may contribute to the prediction of clozapine response [15]. In a study, Vogt *et al.* [16] performed a systematic mutation scan of the complete coding region and splice junction of the 5-HT$_6$ receptor gene to explore the contribution of this gene to the development of bipolar affective disorder and schizophrenia. Investigating 137 unrelated individuals (including 45 bipolar affective patients, 46 schizophrenic patients and 46 unrelated controls) and comparing frequencies between patients and controls, the authors claimed a significant over-representation of the 267C allele among bipolar patients (p = 0.023 not corrected for multiple testing). This finding was followed up in independent sample of 105 bipolar family trios using a family based association design. Fifty-one transmissions could be examined and alleles 267C and 267T were transmitted to the affected offspring in 30 and 21 cases, respectively. The authors claimed that these preliminary data suggest that bipolar affective disorder may be associated with variation in the 5-HT$_6$ genes and it will be important to extend the present analysis to larger samples [16].

The advent of pharmacologically selective 5-HT$_6$ receptor ligands has allowed experimental confirmation of previous experimental outcomes from antisense studies supporting the role of the 5-HT$_6$ receptor in the control of central cholinergic function. Cholinergic involvement in mediating 5-HT$_6$ receptor function was also suggested in a behavioural study of rats unilaterally-lesioned with 6-hydroxydopamine. Unlike L-DOPA or amphetamine, Ro 04-6790 did not cause rotational behaviour in these rats. However, Ro 04-6790 did attenuate scopolamine and atropine-induced circling behaviour in a dose related manner. Reproduction of these effects using SB-271046 was not successful, suggesting that the exact nature of the 5-HT$_6$ receptor/cholinergic interaction still demands further resolution. In a separate study, the acetylcholinesterase inhibitor, physostigmine elicited yawning in rats, which was modestly potentiated by SB-271046. There is clearly a need to further investigate the involvement of the 5-HT$_6$ receptor in this behavioural syndrome (yawning) using a wider range of antagonists from different structural classes. Nevertheless, recent co-localisation studies in rat brain seem to suggest that the 5-HT$_6$ receptor regulation of central cholinergic transmission is not via direct dis-inhibition of central cholinergic neurons *per se*, but through dis-inhibition of GABAergic neurons [17-19].

Despite the conflicting results obtained in the behavioural syndrome studies (thought to be mediated by 5-HT$_6$ receptors), there are some areas of concurrence in the study of 5-HT$_6$ receptor pharmacology. The cognition-enhancing properties of SB-271046 and SB-357134 were investigated in the Morris water maze test of spatial learning and memory in



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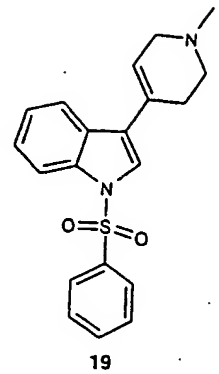

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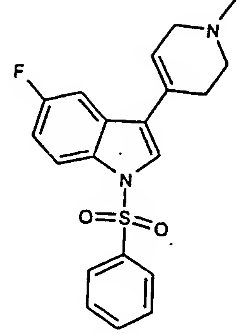

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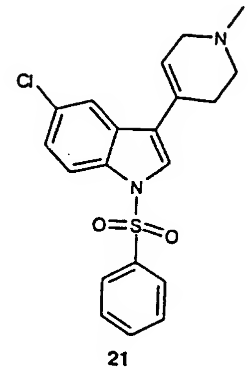

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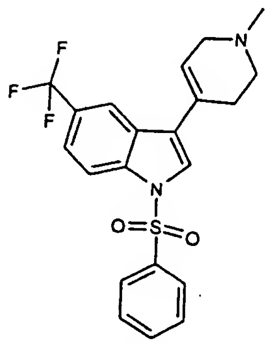

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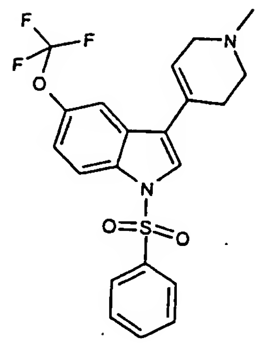

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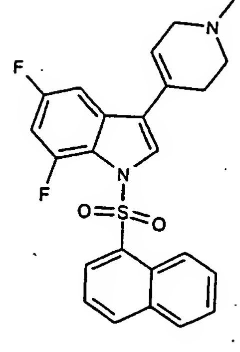

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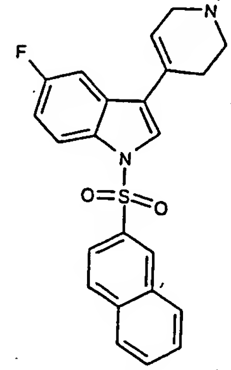

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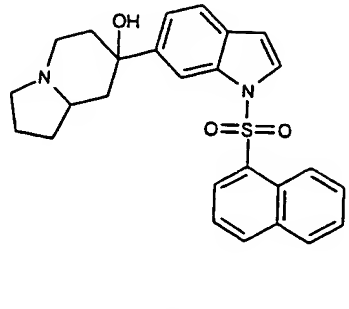
administration, $t_{½}$ = 91 ± 6 min, steady-state volume of distribution = 1.2 ± 0.2 l/kg following i.v. bolus administration and oral bioavailability = 17 ± 9% following po. administration in male Sprague-Dawley rats.

Similarly, in another patent application the same group claimed a series of novel piperidine indole derivatives represented by the general formula 18 and having human 5-HT_6 receptor affinity. This class of compounds was claimed for the treatment of a variety of CNS diseases, including AD, Huntington's chorea, schizophrenia, cognitive disorder and manic depression [31,102]. SAR studies show that the nature of substitution of the indole moiety at the 5-position does not affect the 5-HT_6 binding activity. In fact, keeping the substitution of the indole nitrogen fixed as the phenylsulfonyl group, substitution at the 5-position of the indole skeleton with R_2 (R_2 groups such as a hydrogen 19, fluoro 20, chloro 21, trifluoromethyl 22, or trifluoromethoxy 23), led to approximately the same 5-HT_6 receptor activity profiles (K_i = 3.1 nM, 2.5 nM, 3.0 nM, 2.2 nM and 4.9 nM, respectively). In contrast, the disubstituted indole derivatives such as, 5,7-difluoro 1-(naphthylsulfonyl) indole 24 led to a decrease in the human 5-HT_6 receptor affinity compared to its mono-substituted analogue 9 (ALX1175) (K_i = 1 and 21 nM, respectively). Furthermore, the 1-naphthylsulfonyl derivative 9 was about 20-fold more potent at the human 5-HT_6 receptor compared to the corresponding 2-naphthylsulfonyl analogue 25 (K_i = 1 nM vs 19.5 nM respectively). In the functional adenylyl cyclase assay, the most potent compound, ALX1175 was found to be a competitive antagonist (IC_{50} = 23.7 ± 4.2 nM) with > 100-fold selectivity over a number of other key receptors. ALX-1175 has good CNS penetration (whole brain vs. plasma ratio of 43.4 ± 5.9 following iv. administration), $t_{½}$ = 60 min and oral bioavailability = 19 ± 0.2%.

Replacement of the piperidine ring in ALX-1175 series with bicyclic-piperidine and bicyclic-piperazine moieties was the subject of another patent application by the NPS research group. The general structure claimed in this patent was exemplified by 26. These compounds were stated to be 5-HT_6 receptor antagonists useful for the treatment of psychosis, schizophrenia, depression, manic depression, neurological and memory disturbances, Parkinson's disease (PD) and amyotrophic lateral sclerosis. Over 50 analogues were exemplified. The compound, 5-fluoro-3-[(8a-*R,S*)-1,2,3,5,8,8a-hexahydroindolizin-7-yl]-1-phenylsulfonylindole 27 and their analogues 6,5-bicyclic-piperazine 28 and 6,6-bicyclic-piperazine 29 were among the specific compounds claimed. The binding affinity of these compounds and their functional activity at the human 5-HT_6 receptor, were assessed *in vitro*. Compound 27 showed > 90% inhibition (at 1 μM) of radioligand binding at the human 5-HT_6 receptor and < 10% binding activity at 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors. Compound 27 was also claimed to antagonise human 5-HT_6 receptor mediated cAMP accumulation in HEK-293 cells [103]. In addition, the NPS chemists designed and synthesised novel azaindole derivatives, as a second generation of the previously mentioned series. The compound 3-(1,2,3,5,8,8a-hexahydroindolizin-7-yl)-1-naph-



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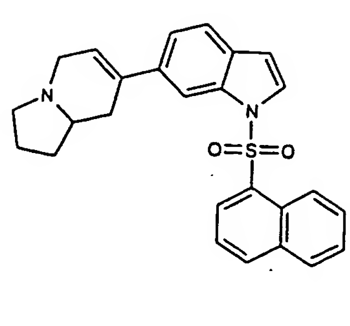

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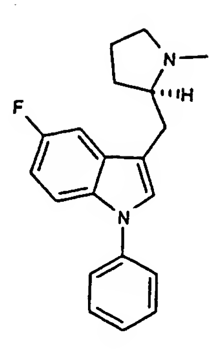

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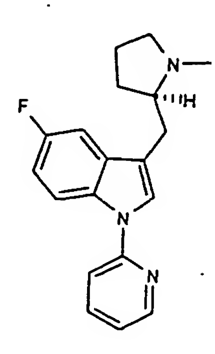

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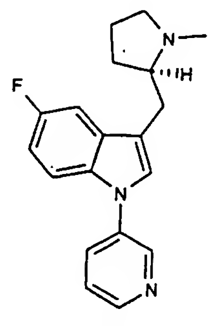

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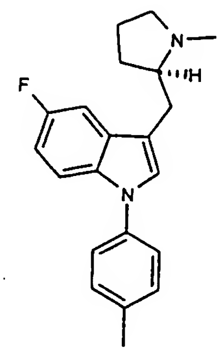

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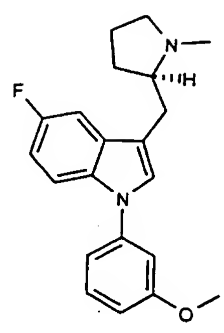

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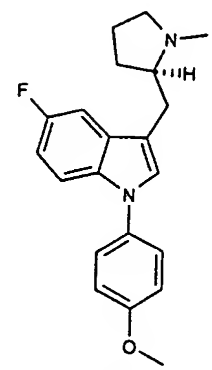

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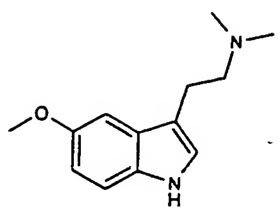
compound 32 was found to be a competitive antagonist (IC_{50} = 7.2 nM), with good selectivity over a number of other key serotonergic and dopaminergic receptors [32,105].

Recently, NPS researchers also published a series of 5-fluoro-(*R*)-3-(*N*-methypyrrolidin-2-yl-methyl)-1-arylindole derivatives as highly potent and selective human $5\text{-}HT_6$ ligands. The SAR showed that, the simple phenyl analogue 40 was a promising initial lead giving a highly potent human $5\text{-}HT_6$ receptor ligand with a K_i value of 2.7 nM and a selectivity of 52.5-fold versus human $5\text{-}HT_7$ receptor. By comparison, the pyridyl analogues 41 and 42 gave greatly reduced potency and selectivity (K_i values of 295 – 147 nM, respectively, and 3.6 – 4.7-fold selectivity vs. $5\text{-}HT_7$). In contrast, substitution on the phenyl ring was tolerated to varying degrees. Substitution with a single methyl residue provided compound 43 with an enhanced human $5\text{-}HT_6$ receptor affinity (compared to 40) and good selectivity over the $5\text{-}HT_7$ receptor. The methoxy residue in the *meta* position (44) or *para* position (45) retained $5\text{-}HT_6$ receptor binding affinity. However, the concomitant reduction in $5\text{-}HT_7$ receptor affinity resulted in an enhanced $5\text{-}HT_7/5\text{-}HT_6$ selectivity (85.5 to 168-fold). The methoxy substituent at the *ortho* position was less favourable, with reduced potency and only moderate $5\text{-}HT_7/5\text{-}HT_6$ selectivity. Substitution with fluoro at the *para* position resulted in enhanced binding ($5\text{-}HT_6$ = 0.34 nM) and good selectivity ($5\text{-}HT_7/5\text{-}HT_6$ = 70.5-fold) but *ortho* substitution decreased binding affinity (3-fold less potent than simple phenyl analogue 40). In contrast to the fluoro and methyl at *para* position analogues that resulted in improvements versus compound 40, the combination of the two groups in the trifluoromethyl analogues resulted in reduction of both potency and selectivity versus compound 40. The nitro group was not well-tolerated, giving results similar to the pyridyl analogues 41 and 42, with reduced potency and selectivity versus compound 40. Of the dimethyl analogues examined, only the 2,3-dimethyl analogue resulted in improved potency ($5\text{-}HT_6$ = 0.87 nM) and selectivity ($5\text{-}HT_7/5\text{-}HT_6$ = 131-fold) over 40. The symmetrical 3,5-dimethyl derivative was least well-tolerated, losing both potency ($5\text{-}HT_6$ = 165 nM) and selectivity ($5\text{-}HT_7/5\text{-}HT_6$ = 9.2-fold) [33]. Similarly, in collaboration with NPS Pharmaceuticals, Prof. Glennon *et al.* have described a series of N1-(benzenesulfonyl)tryptamine derivatives as novel human $5\text{-}HT_6$ receptor antagonists. N1-Benzenesulfonamido-5-methoxy-*N,N*-dimethyltryptamine 46 binds to the human $5\text{-}HT_6$ receptor with higher affinity (K_i = 2.9 ± 0.4 nM) than that of 5-HT (47, K_i = 78 ± 6 nM) itself. Replacement of the N_1-benzenesulfonamido group in compound 46 with the sterically larger 2-naphthalene-sulfonamido group 48, 1-naphthalenesulfonamido group 49 or 2,5-dimethoxyphenyl 50 also had little effect (K_i = 1.6 ± 0.3 nM and K_i = 0.9 ± 0.2 nM, respectively). Moving the 5-methoxy substituent of 46 to the 4-, 6- and 7-positions led to analogues 51, 52 and 53, respectively. With the exception of the 7-methoxy derivative 53 (K_i = 240 ± 32 nM, ~ 170-fold reduction in affinity), the $5\text{-}HT_6$ affinity was decreased only by ~ 4-fold when compound 50 (K_i = 1.3 ± 0.2 nM) is compared with 51 (K_i = 7.4 ± 0.6 nM) and 52 (K_i = 9.5 ± 0.6 nM). Given the high affinity of compound 46 for the human $5\text{-}HT_6$ receptor, the binding of this compound was examined at several other serotonin receptor populations and it was found to have > 100-fold selectivity over serotonin receptors, such as $h5\text{-}HT_{1A}$, $h5\text{-}HT_{1B}$, $h5\text{-}HT_{1E}$, $h5\text{-}HT_3$ and $h5\text{-}HT_7$. In contrast, 46 displayed high affinity for rat



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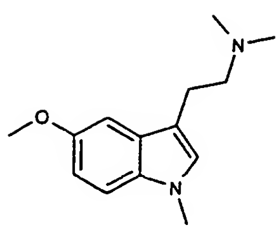

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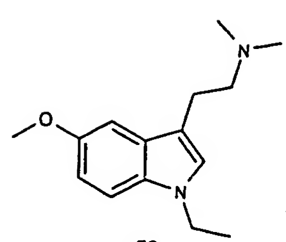

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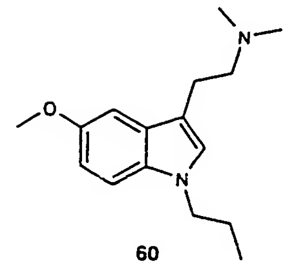

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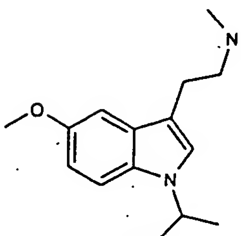

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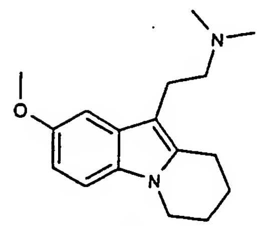

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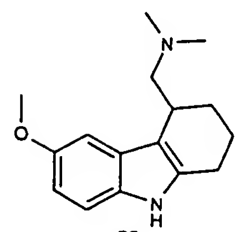

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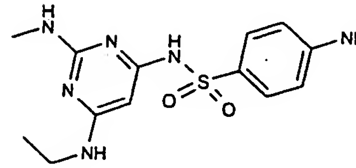

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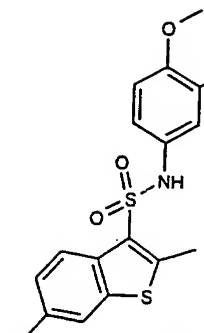
(K_i = 1030 nM). Finally, the 2-ethyl group in 57 was tethered to afford compound 63. Compound 63 was found to bind to the 5-HT_6 receptor with ~ 3-fold lower affinity than 55 (K_i = 168 nM) [35-37,106].

3.2 Hoffmann-La Roche

Hoffmann-La Roche researchers were the first to identify potent and selective 5-HT_6 receptor antagonists exemplified by the two lead compounds 4-amino-*N*-(2,6-*bis*-methylamino-pyrimidin-4-yl)-benzene sulfonamide Ro 04-6790 and 4-amino-*N*-(2,6-*bis*-methylamino-pyridin-4-yl)-benzene sulfonamide Ro 63-0563. These two compounds were found to have reasonable affinity for the rat 5-HT_6 receptor (pK_i = 7.8 and 7.9, respectively) and > 100-fold selectivity over other receptor sites. Furthermore, these compounds behaved as competitive antagonists, causing a parallel shift in the dose response curve to 5-HT, and had no effect on the basal level of cAMP, suggesting that they are antagonists at the 5-HT_6 receptor [4,38]. Ro 04-6790 was sufficiently brain penetrant that a dose of 30 mg/kg was predicted to occupy > 70% of 5-HT_6 receptors. Ro 63-0563 was radiolabelled with tritium in positions 3 and 5 of the benzene ring and used to label both the rat and human recombinant receptor systems [39]. Specific binding of [^{3}H]-Ro 63-0563 to recombinant rat and human 5-HT_6 receptor was saturable, rapid and reversible with respective equilibrium dissociation constant or K_d values of 6.8 and 4.96 nM. The pharmacological profile of both receptors radiolabelled with [^{3}H]-Ro 63-0563 was similar to that obtained with either [^{3}H]-LSD or [^{3}H]-5-HT. Recently, Bös *et al.* published the SAR within the Ro 04-6790 and Ro 63-0563 series. It has been shown that the ethyl group at the amino substituent 64, as well as small rings such as azetidine 65 and pyrrolidine 66 in position 2, gave compounds with similar 5-HT_6 receptor affinities compared to the lead compound 12. The introduction of larger groups or no substitution at the amino group in this position, led to ligands with reduced 5-HT_6 receptor affinity. It was claimed that omitting the 4-amino functionality of the lead compound 12 or replacing it by other substituents, such as halogen or alkyl groups, resulted in a loss of 5-HT_6 receptor affinity. Replacement of the pyrimidine derivative with a pyridine 13 increased binding affinity (pK_i = 7.8). The bromo substituted compound 67 showed a decrease in human 5-HT_6 receptor affinity, with a concomitant increase in compound lipophilicity (pK_i = 7.3 and logD = 1.7, compared to 7.8 and 0.03 for 13). Replacement of the heterocyclic nucleus with a simple phenyl ring produced ligands with high affinities for the 5-HT_6 receptor. For example, for the bromo-amino (68) and methoxy-amino (69) derivatives bind with affinities (pKi) of 7.7 and 7.8, respectively. Incorporation of the amino nitrogen of this series of ligands into a 4-sulfamoylsubstituted indole led to potent 5-HT_6 receptor antagonists, such as 4-(4-aminobenzylsulfonyl)-6-bromo-1H-indole 70 (pK_i = 7.3 and logD = 1.77) [40-42,107]. Similarly, in another patent application the same team claimed a series of novel pyrazolopyrimidine and pyrazolotriazine compounds with 5-HT_6 receptor affinity. These ligands were claimed to be suitable for the treatment and prevention of AD, Huntington's chorea, motor neuron disease, PD, psychosis, schizophrenia and depression. 3-(benzenesulfonyl)-5-methyl-2-(methylthio)pyrazolo[1,5-a]pyrimidin-7-amine (71) was one of the compounds specifically claimed. Standard assay methods were used to determine 5-HT_6 receptor binding affinity. The compounds exhibited pK_i values in the range 6.5 – 9.5. However, no specific data were disclosed [108].

Ro 04-6790 was the first potent, selective 5-HT_6 receptor antagonist used in behavioural studies [38,43]. As a confirmation of the *in vivo* activity of this compound, Woolley *et al.* recently published a study investigating the effect of intracerebroventricular administration of 5-HT_6 antisense oligonucleotide (5-HT_6 AO) complementary to bases 1 – 18 of the rat cDNA initiation sequence (1.5 mg b.i.d. for six days) and i.p. injection of Ro 04-6790 (10 or 30 mg/kg once-daily for three



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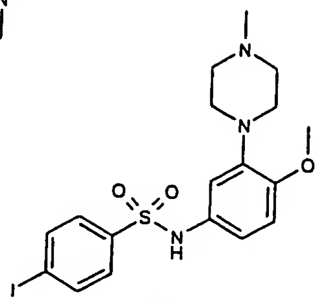

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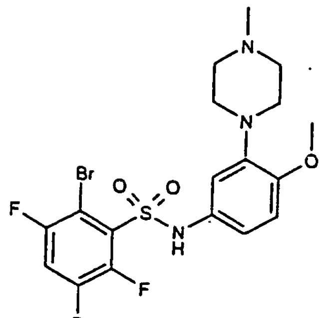

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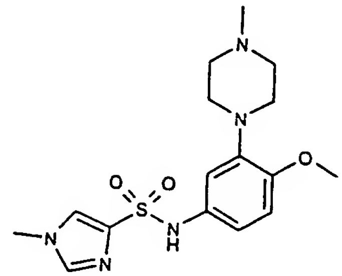

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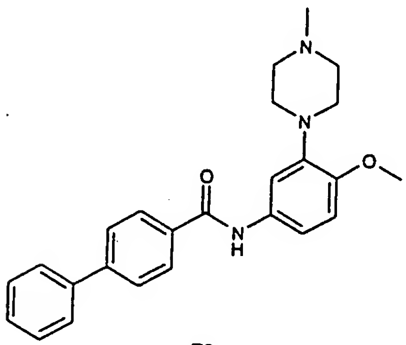

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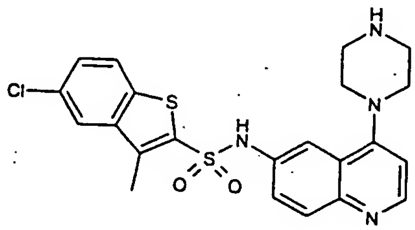

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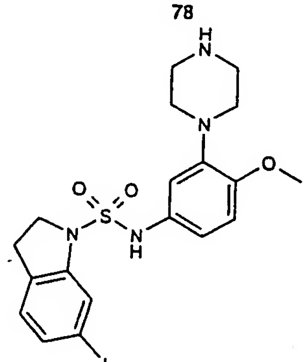

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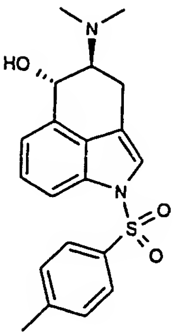
mide 79 was among the 13 compounds specifically claimed. Although, no biological data were disclosed, these compounds were stated to show pK_i values of > 7.0 [114]. The outcome of these medicinal chemistry efforts led to the 5-chloro-3-methylbenzothiophene 73 as the optimal compound for further evaluation. Pharmacokinetic studies of compound 73 at steady-state in rat following a 16 h infusion demonstrated that, it was moderately brain penetrant (18%) and had relatively low blood clearance compared to 73 (12.5 ml/min/kg vs. 60 ml/min/kg). However, in rats, compound 73 was metabolically labile, undergoing N-demethylation to the corresponding NH-piperazine analogue SB-271046. The receptor binding profile of SB-271046 showed a slightly reduced human 5-HT_6 affinity relative to the N-methylpiperazine derivative 73 (pK_i = 8.9). SB-271046 was shown to be a competitive antagonist with a pA_2 value of 8.7. Furthermore, SB-271046 was found to be highly selective (> 200-fold) against a battery of > 50 receptors, enzymes, or ion channels. Pharmacokinetic studies demonstrated this metabolite to be moderately brain penetrant (10%), subject to low blood clearance (7.7 ml/min/kg) with a good half-life in rats (4.8 ± 0.1 h) and had excellent oral bioavailability (F = 80%). The replacement of the (4-methoxy-1-piperazinyl)phenyl moiety in 73 by quinoline substituted at the 4-position 80, yielded a similar binding affinity for the 5-HT_6 receptor (pK_i = 8.7) [47]. In an attempt to increase the brain penetration of these compounds, further SAR development in the piperazine-benzenesulfonamide series (i.e., replacing sulfonamide NH with more lipophilic groups) led to the discovery of conformationally restricted indoline analogues 81 and 82 with high affinity for the 5-HT_6 receptor (pK_i = 9.5 and 8.4, respectively). Similar conformational analogues, such as the tetrahydroquinoline and isoquinoline derivatives 83 and 84 also possessed good 5-HT_6 receptor affinity (pK_i = 9.5 and 9.3, respectively). However, compounds from this series, such as 82 and 84, had *in vivo* clearance in rats ≥ liver blood-flow [48,115]. These efforts also led to discovery of the [^{125}I] radiolabelled compound 4-iodo-*N*-[4-methoxy-3-(4-methypiperazin-1-yl)phenyl]benzenesulfonamide 76 ([^{125}I]SB-258585). In addition to [^{3}H]-Ro 63-0563, [^{125}I]SB-258585 is an important tool for helping the scientific community in further understanding the therapeutic benefits of the 5-HT_6 receptor [39,49].

3.4 Merck

Through internal screening of their sample collection, Merck recently reported their discovery that N-(arylsulfonyl)indole derivatives were potent, selective human 5-HT_6 receptor ligands [50,116]. This finding was previously reported by Glennon *et al.* in collaboration with NPS pharmaceuticals. One lead candidate, compound 46, was found by both groups to be a potent and selective human 5-HT_6 receptor antagonist. Similar results were also obtained upon profiling this compound against a number of serotonin and dopamine receptors, demonstrating that 46 exhibited considerable affinity for the 5-HT_2 receptor (K_i at 5-HT_2 was 65 nM). However, the Merck researchers then determined whether compound 46 penetrated the brain and interacted with the serotonin receptors by using head twitch response elicited by 5-HT_2 receptor agonist, such as mescaline and DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane], that is selectively blocked by selective 5-HT_2 receptor antagonists. In this



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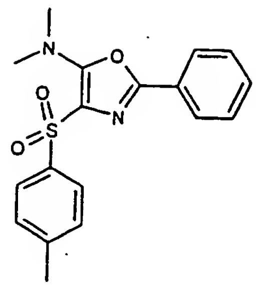

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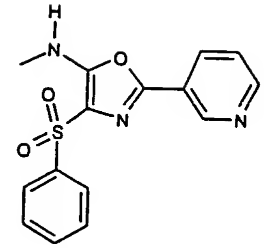

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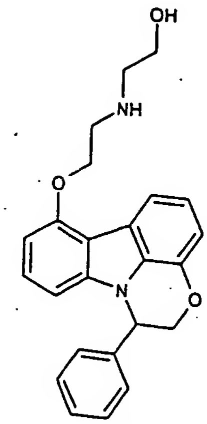

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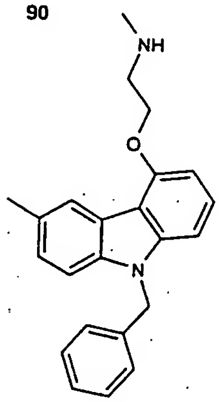

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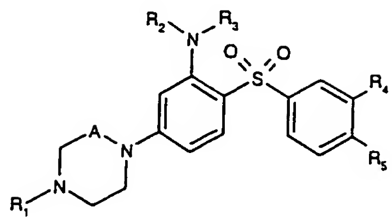

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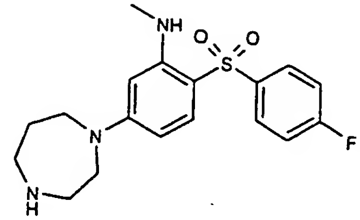

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of those ligands. The binding affinities (K_i values) were in the range 1.1 - 97 nM. The specific compound 2-[2-(1-phenyl-1,2-dihydro[1,4]oxazino[2,3,4,jk]carbazol-7-yloxy)ethylamino]ethanol 93 is one of 22 compounds specifically claimed, having a K_i value of 1.1 nM [119]. The second patent application disclosed a family of aminoalkoxy carbazoles as 5-HT_6 receptor ligands. Similarly, the compounds were assessed for their *in vitro* 5-HT_6 receptor binding activity and showed K_i values ranging from 2.2 nM to 482 nM. The specific compound, N-[2-(9-benzyl-6-methyl-9H-carbazol-4-yloxy)-ethyl]-*N*-methylamine 94, was one of 62 analogues specifically claimed and had a K_i value of 2.2 nM [120].

During the preparation of this review, a third patent application was published from Pharmacia & Upjohn on a series of bis-arylsulfone derivatives exemplified by the general structure 95. These compounds were claimed to be useful for the treatment of diseases in which the 5-HT receptors, particularly the 5-HT_6 receptor, is implicated such as anxiety, depression, schizophrenia, obsessive/compulsive disorder, migraine, addiction, obesity, eating disorders, sleep disorders and numerous other CNS diseases. These compounds were stated to be 5-HT_6 receptor ligands, which selectively bind to the 5-HT_6 receptor. The specific compound 5-(1,4-diazepan-1-yl)-2(4-fluorophenylsulphonyl)-*N*-methylaniline 96 is one of 222 compounds specifically claimed. The K_i value for the corresponding hydrochloride was found to be 1.4 nM [121].

4. Expert opinion

The 5-HT_6 receptor was identified and characterised using molecular biological techniques and evidence is accumulating that this receptor mediates specific functions. Based on the aforementioned evidence, it is now becoming clear that targeting the 5-HT_6 receptor with selective antagonists is a viable drug development strategy, since novel drugs with potential for treating a large number of common disorders, including schizophrenia and cognitive dysfunction, are possible. Furthermore, the CNS specific localisation of this receptor makes this target very attractive for the treatment of CNS disorders with little likelihood of peripheral side effects. As a result, the identification of potent, selective and structurally diverse 5-HT_6 receptor antagonists, such as those described above, should give researchers confidence for investigating whether the observed pharmacological effects are due to 5-HT_6 receptor antagonism or to the properties of a particular compound. In addition, the growing number of novel selective 5-HT_6 receptor ligands from the different structural classes should also help in resolving the lack of agreement in previous animal experiments. The ongoing drug discovery efforts geared toward the development of novel 5-HT_6 receptor antagonists can potentially usher in a new generation of drugs with enhanced efficacy and reduced side effects. The development of these drugs may revolutionise the treatment of a number of common CNS disorders.

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SB-271046 SmithKline Beecham

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Current Opinion in Investigational Drugs 2001 2(1):118-122

SmithKline Beecham is developing the 5-HT$_6$ antagonist, SB-271046, as a potential cognition enhancer. By December 1999, phase I trials had commenced [360354]. This drug was originally being developed primarily for the treatment of schizophrenia [284490], however, cognitive disorders, including but not limited to Alzheimer's disease, have been the main target since 1998 [394309].

SB-271046 is a potent, selective 5-HT$_6$ antagonist with a pK$_i$ value of 8.9 [333710].

SB-258585, also known as 4-iodo-N-[4-methoxy-3-(4-methyl-piperazin-1-yl)phenyl]benzenesulfonamide is an analog of SB-271046 [322488].

Data recently presented at the Society for Neuroscience annual meeting in November 2000 demonstrated that administration of SB-271046 resulted in a significant increase in glutamate and aspartate levels in the frontal cortex, without affecting noradrenaline, dopamine or 5-HT levels. This was stated to suggest that 5-HT$_6$ antagonists might therefore be useful for treating cognitive dysfunction [390469]. The drug has also been radiolabeled in order to provide an assay for estimating in vivo 5-HT$_6$ receptor occupancy [390470].

Originator SmithKline Beecham plc

Status Phase I Clinical

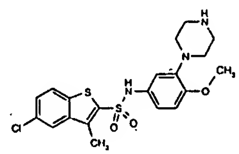
Indication Schizophrenia

Action 5-HT$_6$ antagonist

Synonyms SB-258585

CAS Benzo[b]thiophene-2-sulfonamide, 5-chloro-N-[4-methoxy-3-(1-piperazinyl)phenyl]-3-methyl-
Registry No: 209481-20-9
Note: SB-271046

CAS Benzenesulfonamide, 4-iodo-N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-
Registry No: 209480-63-7
Note: SB-258585



Introduction

Since atypical antipsychotics, and some antidepressants, have relatively high affinities for certain subtypes of serotonin (5-HT) receptors, there has been an improved effort to find new compounds with high selectivity and affinity for these receptors. It is hoped that compounds discovered by such a strategy could be utilized in the treatment of psychiatric disorders [345797]. Amongst the numerous subtypes of 5-HT receptors, the 5-HT$_6$ subtype has recently attracted special attention, since some of the most effective antipsychotics (such as clozapine), and some antidepressants, demonstrate high affinity for this receptor subtype, where they act as antagonists [389841].

5-HT$_6$ receptors are present at high levels in key structures of the forebrain, such as the cortex, caudate/putamen, nucleus accumbens, and hippocampus [333710]. Moreover, a role for these receptors in memory and cognition was suggested when it was found that administration of antisense oligonucleotides directed to mRNA encoding the 5-HT$_6$ receptor induces a behavioral syndrome that is blocked by the muscarinic antagonist, atropine [389843]. Accordingly, it was suggested that 5-HT$_6$ receptor antagonists might be useful for the treatment of memory and cognitive dysfunction.

The first selective antagonists developed for the 5-HT$_6$ receptor were Ro-04-6790 and Ro-63-0563 (F Hoffman-La Roche Ltd), which both had moderate affinity for the receptor. As expected, they also appeared to enhance cholinergic neurotransmission [383182]. However, these compounds had poor brain penetration. Further development of selective antagonists led to SB-271046, which has both high affinity and high selectivity for 5-HT$_6$ receptors, high oral bioavailability and brain penetration compared with the early agents.

Synthesis and SAR

SB-271046, also known as 5-chloro-3-methyl-benzo[*b*] thiophene-2-sulphonic acid (4-methoxy-3-piperazin-1-yl-phenyl)amide monohydrochloride, was obtained after studies performed on a variety of compounds screened against cloned human 5-HT$_6$ receptors [315662]. One of these compounds, 4-bromo-*N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]benzenesulfonamide, showed high affinity (pK$_i$ = 8.3) for the 5-HT$_6$ receptor and a 50-fold greater selectivity at a number of other receptors, including ten different 5-HT receptor subtypes. In order to study SAR related to SB-214111 (SmithKline Beecham plc), 4-methoxy-3-(4-methylpiperazin-1-yl)-aniline was coupled with various sulfonyl chlorides encompassing several different aromatic nuclei. Among them, the 5-chloro-3-methylbenzothiophene derivative was identified as the most potent (affinity pK$_i$ = 9.2) and selective compound (300-fold more selective for 5-HT$_6$ receptor than for 13 other receptor types). This compound was metabolically N-dealkylated in rats to produce a derivative which was found in blood at significant levels, and had a structure corresponding to that of SB-271046. Subsequently SB-271046 was synthesized via the BOC-protected piperazine.

Pharmacology

SB-271046 binds with great affinity to the serotonin 5-HT$_6$ receptor (pK$_i$ = 8.9 for human receptors; pK$_i$ = 9.3 for rat receptors) and showed good selectivity for this receptor (> 200-fold) compared to more than 54 receptors, enzymes and channels [334508]. In a functional adenylate cyclase assay with HeLa cell membranes [315662], SB-271046 was a competitive antagonist (pA2 = 8.7). The compound demonstrated no significant inhibition of the major human P450 enzymes *in vitro*. In the rat, pharmacokinetic studies showed that SB-271046 has a brain penetration of 10%, low blood clearance (7.7 ml/min/kg) and an oral bioavailability > 80% [315662]. In an *ex vivo* study with homogenates of brain striatum from rats treated *in vivo* with 0.1 to 100 mg/kg of SB-271046, binding of the specific, radiolabeled 5-HT$_6$ receptor ligand, [^{125}I]SB-258585, was prevented with ED$_{50}$ = 30 mg/kg [339415], [346161], [382544], [389849].

In vivo effects of SB-271046 on brain neurochemistry were recently studied by Dawson *et al* using microdialysis from the striatum and frontal cortex in the freely moving rat [378931]. SB-271046 (10 mg/kg) did not change the concentrations of 5-HT, dopamine or noradrenaline in any of the regions studied. Concentrations of aspartate and glutamate remained also unchanged in the striatum. However, SB-271046 produced increases in glutamate (> 3-fold) and aspartate (> 2- fold), as measured in the cortex. This effect was blocked by tetrodotoxin, a sodium channel blocker, suggesting that SB-271046 induces the release of glutamate and aspartate from a neuronal population in the cortex. As yet, there is no evidence to suggest an interaction of SB-271046 on glutamate transporters. Consequently, the authors of the study speculate that SB-271046 enhances excitatory neurotransmission by blocking tonic serotonergic inhibition of cortical excitatory afferents.

The localization of the 5-HT$_6$ receptors responsible for the actions of SB-271046 and its analogs is most likely post-synaptic, since autoradiography, immunohistochemical and mRNA *in situ* hybridization show that the receptor appears to be near to the site of protein synthesis (somata and dendrites) [379025], [389841]. In addition, dendritic localization of 5-HT$_6$ receptors in the striatum and dentate gyrus has been demonstrated in the rat [391679]. Since 5-HT$_6$ receptor mRNA has not yet been identified in the raphe, this suggests that 5-HT$_6$ receptors are not found presynaptically on serotonergic neurons but post-synaptically on target neurons, eg, in the striatum and dentate gyrus. It remains possible that 5-HT$_6$ receptors may be heteroreceptors on serotonergic terminals.

The expression level of the immediate early gene, c-fos, is affected by antipsychotics. One study compared the expression of Fos-like immunoreactivity after treatment with SB-271046, clozapine or haloperidol [379022]. Only haloperidol and clozapine produced a significant increase in Fos-like immunoreactive structures in the striatum, while SB-271046 did not produce any change. The reason for this lack of c-fos activation is unknown as it suggests that any putative antipsychotic or cognitive effects of SB-271046 do not involve changes in c-fos expression levels.

SB-271046 also presents anticonvulsant effects, as assessed in the rat maximal electroshock threshold test [322488], [378931]. SB-271046 produced an increase in seizure threshold over a range of doses (0.1 to 30 mg/kg po, 2 h before testing), with a minimal affective dose of 0.1 mg/kg. At 10 mg/kg, the effect was sustained up to 8 h. No evidence of tolerance to the anticonvulsant activities of SB-271046 was observed following repeated administration at 10 mg/kg bid for 7 days. No behavioral side effects were noticed. It was concluded that SB-271046 produced potent and long-lasting anticonvulsant activity, although the magnitude of this effect was modest in comparison to that of known anti-epileptic drugs, such as carbamazepine, evaluated in the same model [322488]. The level of anticonvulsant activity correlated with the concentration of SB-271046 in blood (EC$_{50}$ = 0.16 μM) and in brain (C$_{max}$ = 0.01 to 0.04 μM) [334513], [385302].

The cholinergic system plays fundamental roles in memory and cognitive functions. Accordingly, two studies to determine the effects of SB-271046 in two rat models of memory and learning were conducted [389855], [319557], [322488]. In the water maze spatial learning task, there was no significant effect of treatment on acquisition of the water maze. However, a repeated measures analysis showed a significant effect of treatment on the percentage of time spent in the platform quadrant and a significant difference between vehicle and 10-mg/kg groups. In a different experiment, using a T-maze spontaneous alternation task in aged rats, effects of SB-271046 on choice accuracy were investigated. At 20 mg/kg, SB-271046 attenuated the deficit in T-maze choice accuracy induced by a 30-s delay.

As discussed previously, the administration of antisense oligonucleotides directed to 5-HT$_6$ receptor mRNA induced a behavioral syndrome that could be blocked by atropine [389843]. In addition, Bourson *et al* reported that, in 6-hydroxydopamine lesioned rats, the 5-HT$_6$ receptor antagonist, Ro-04-6790 (F Hoffman-La Roche Ltd), inhibited rotational behavior induced by the muscarinic antagonists, scopolamine and atropine [391714].

Since 5-HT$_6$ receptor activation appears to regulate the cholinergic system, the effects of SB-271046 on yawning were investigated in rats [334508]. This compound had no effect on yawning *per se*. However, SB-271046 (10 mg/kg po) enhanced the increased yawning produced by physostigmine (0.3 mg/kg ip).

Metabolism

SB-271046 demonstrated no significant inhibitory activity at the major human P450 enzymes *in vitro*. In the rat, pharmacokinetic studies showed that SB-271046 has a brain penetration of 10%, low blood clearance (7.7 ml/min/kg) and an oral bioavailability > 80% [315662].

Toxicity

No toxic effects have been described to date in the animal tests performed with SB-271046. In a rat maximal electroshock seizure threshold test of the anticonvulsant properties of SB-271046, no behavioral depressant action was observed [334513].

Clinical Development

Phase I

Trials in volunteers had started by December 1999, but no data are currently available [360354].

Side Effects and Contraindications

No data are currently available.

Current Opinion

In animal models, SB-271046 demonstrates specific cognitive effects. These effects appear to be mediated by 5-HT$_6$ receptor-induced changes in levels of glutamate and acetylcholine or in the responses of their receptors, while levels of dopamine, 5-HT and norepinephrine undergo little or no change. This specificity of action, without other apparent side or toxic effects, may prove very valuable for treating cognitive abnormalities in schizophrenia and neurodegenerative diseases. The specificity of action, however, would also predict that antagonists of 5-HT$_6$ receptors would have less effect on other aspects of the pathophysiology of schizophrenia or other cognitive disorders related to alterations in monoamines. However, given the complexity of human emotional and cognitive functions, only clinical trials with SB-271046 will provide the evidence necessary for understanding the influence of 5-HT$_6$ in the various higher functions of the human brain.

Development history

Developer	Country	Status	Indication	Date	Reference
SmithKline Beecham plc	UK	C1	Schizophrenia	27-MAR-00	360354

Literature classifications

Chemistry

Study Type	Result	Reference
Synthesis and SAR	SB-271046 is the N-demethylated form of the 5-chloro-3-methylbenzothiophene derivative of 4-bromo-*N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]enzenesulfonamide.	315662, 334508
SAR	Cis orientation of the aromatic rings around the sulfonamide is preferred.	338560
SAR	Sulfonamide linkage is better than an amide moiety.	338560
SAR	The piperazine ring interacts with an aspartic acid residue in transmembrane loop III of the 5-HT$_6$ receptor.	338560

Biology

Study Type	Effect Studied	Experimental Model	Result	Reference
In vitro	Receptor selectivity.	Radioligand binding assay.	The ^{125}I derivative of SB-258585 (4-[^{125}I]phenylsulfonyl analog of SB-271046) is highly selective for 5-HT$_6$ receptors.	315662
In vitro	Receptor binding properties.	Radioligand binding assay.	Radioactive analog, SB-258585, has high affinity for recombinant human 5-HT$_6$ receptor expressed in HeLa cell lines.	322488
In vitro	Localization of 5-HT$_6$ receptors.	Binding of radioactive analog analog, SB-258585, to brain sections.	High levels in the cerebral cortex, nucleus accumbens, caudate-putamen and CA1/dentate gyrus of the hippocampus, and at moderate levels in the substantia nigra and thalamus.	333710
In vivo	Yawning.	Physostigmine-induced (1, 3, 10 mg/kg po) yawning in rats.	Significant increase in induced yawning at 10 mg/kg.	322488, 334508
In vivo	Anticonvulsant activity.	Rat maximal electroshock seizure threshold test. Doses of 0.1 to 30 mg/kg.	Dose-dependent elevation of seizure threshold (MED = 0.1 mg/kg). At 10 mg/kg, effect was sustained up to 8 h.	334513, 385302
In vivo	c-Fos expression.	Administration of SB-271046, 10 mg/kg po.	No changes in c-Fos immunoreactivity.	379022
In vivo	Memory (retention).	The water maze test in rats.	Improved retention with 10 mg/kg dose.	389855
In vivo	Learning.	Operant delayed alternation task by aged rats.	Improvement in choice accuracy at 1 mg/kg.	389855
In vivo	Levels of serotonin (5-HT), dopamine (DA), noradrenaline (NA), glutamate (GL), and aspartate (AS).	Microdialysis of freely moving rats in striatum and frontal cortex.	No change in basal levels of 5-HT, NA or DA in any region. No change in GL or AS in striatum. Tetrodotoxin-dependent increase of GL (82.3%) and AS (62.1%) in frontal cortex.	378931
Ex vivo	Binding inhibition of SB-258585.	*Ex vivo* binding assay.	SB-271046 and SB-214111 inhibited the binding of SB-258585 with ED_{50} = 30 mg/kg.	382544

Metabolism

Study Type	Effect Studied	Experimental Model	Result	Reference
In vivo	Oral bioavailability.	Oral administration in rats.	Oral bioavailability > 80%.	315662
In vivo	Half life.	Oral administration in rats.	$T_{1/2}$ = 4.8 h.	315662
In vivo	pA2.	Oral administration in rats.	pA2 = 8.7.	315662
In vivo	Brain penetration.	Oral administration in rats.	Moderate (10%).	315662
In vivo	Brain:blood ratio.	Oral administration in rats.	Ratio of 0.1.	338560
In vitro	Cytochrome P450 inhibition.	Human microsomal preparations.	No inhibition of cytochrome P450.	338560

Associated patent

Title Sulfonamide derivatives with 5-HT$_6$ antagonist activity for treating CNS disorders.

Assignee SmithKline Beecham plc

Publication WO-09827081 25-JUN-98

Priority GB00026377 19-Dec-96

Inventors Bromidge SM, King FD, Wyman PA.

Associated references

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315662 5-Chloro-N-(4-methoxy-3-piperazin-1-ylphenyl)-3-methyl-2-benzothiophenesulfonamide (SB-271046): a potent, selective and orally bioavailable 5-HT$_6$ receptor antagonist Bromidge SM, Brown AM, Clarke SE, Dodgson K, Gager T, Grassam HL, Jeffrey PM, Joiner GF, King FD, Middlemiss DN, Moss SF *et al J MED CHEM* 1999 42 2 202 - 205

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M-100907 Aventis

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M-100907 is a highly selective 5-HT$_{2A}$ antagonist that is being developed by Aventis Pharmaceuticals, formerly Hoechst Marion Roussel (HMR), for the potential treatment of schizophrenia. M-100907 is in phase III trials for chronic schizophrenia [307936], [307942], [307940]. In August 1999, development was discontinued for acute schizophrenia (schizoaffective disorder) on the basis of poor results [335083].

M-100907 is a potent antagonist in every putative animal behavioral model of schizophrenia that involves activation of 5-HT$_{2A}$ receptors [181713]. Interestingly, M-100907 is also active in animal models involving blockade of NMDA glutamatergic channel receptors, an effect known to resemble some behavioral symptoms of schizophrenia in man [390328].

M-100907 belongs to a series of piperidine derivatives, which were originally disclosed in the associated patent, EP-00208235. M-100907 is specifically claimed in a later patent, EP-00531410. This patent describes superior in vivo potency for M-100907 and its claims include the use of M-100907 for the treatment of thromboembolic disorders. The use of M-100907 for the treatment of various developmental neurological disorders such as autism and attention deficit hyperactivity disorder is disclosed in WO-09956750.

In 1996, this product was designated one of HMR's nine top priority products, serving an unmet medical need and addressing a potential market in excess of US $500 million per year [221118]. In January 1999, BT Alex Brown predicted sales of US $30 million in 2000 rising to US $220 million in 2002 [318220]. In April 1999, ABN Amro predicted annual sales of DM 50 million in 2000, rising to DM 150 million in 2002 [328676].

Originator Aventis Pharmaceuticals Inc

Status Phase 3 Clinical

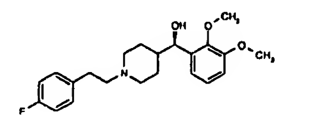
Indication Psychosis, Schizoaffective disorder, Schizophrenia

Action 5-HT$_{2A}$ antagonist

Synonyms MDL-100907, MDL-101860, MDL-28161, MDL-100151, MDL-105725, MDL-100009

CAS 4-Piperidinemethanol, α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-, (R)-
Registry No: 139290-65-6
Note: M-100907

CAS 4-Piperidinemethanol, 1-[2-(4-fluorophenyl)ethyl]- α-(3-hydroxy-2-methoxyphenyl)-, (αR)-
Registry No(s): 189192-18-5
Note: MDL-105725 - active metabolite



Introduction

For over 35 years, derivatives of chlorpromazine (phenothiazines) and haloperidol (butyrophenones) have been used successfully to treat psychotic behaviors, including schizophrenia. The exact mechanism of action of these antipsychotic agents remains to be elucidated, and many hypotheses have been proposed and tested in animal models. To date, the only reliable predictor of antipsychotic activity is the ability of an agent to inhibit the dopamine D$_2$ receptor [390342]. Unfortunately, this activity also correlates with incidences of extrapyramidal side effects (EPS) in man. With the observation that atypical antipsychotic agents also bind more potently to the 5-HT$_{2A}$ receptor, particularly *in vivo* [200641], it was suggested that this would lead to a lower propensity for causing EPS in man [1857]. This hypothesis is refuted by the fact that neither ketanserin nor ritanserin (Janssen Pharmaceutica NV) were able to reverse haloperidol-induced catalepsy in rats [390327], [390334], nor were schizophrenic patients free from experiencing EPS while taking olanzapine (Zyprexa; Eli Lilly & Co) or risperidone (Risperdal Janssen Pharmaceutica NV) in doses that caused high dopamine D$_2$ receptor occupancy and concomitant high 5-HT$_{2A}$ receptor occupancy [390321]. However, the idea that 5-HT$_{2A}$ receptor antagonism alone could convey antipsychotic activity [352899], perhaps through glutamate-mediated control of dopamine release [353639], [378994], was born [181713]. A definitive answer to this question requires an agent that selectively blocks the 5-HT$_{2A}$ receptor without inhibiting other neurotransmitter receptors. M-100907 is such an agent [390339].

Synthesis and SAR

The racemic desfluoro analog of M-100907 (MDL-26508) was synthesized in 1984 by Albert Carr and Norbert Wiech at Aventis (previously known as Merrell-Dow, then Hoechst-Marion Roussel [350762]) in Cincinnati (US-05169096). Two different routes for producing racemic M-100907 (MDL-100151) have been reported: (i) Ethyl isonipecotate is *N*-alkylated with 4-fluorophenethyl bromide and the product is treated with *N,O*-dimethylhydroxylamine and ethylmagnesium bromide [390318]. Reaction with the lithium salt of veratrole and reduction with sodium borohydride gives MDL-100151; (ii) isonipecotic acid is alkylated with di-tert-butyl dicarbonate and the resulting product is condensed with *N,O*-dimethylhydroxylamine to give the BOC-protected 4-(*N*-methoxy-*N*-methylcarboxamido)-1-piperidine. Reaction with the lithium salt of veratrole as